Observation of the DNA ion-phosphate vibrations

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Abstract

The low-frequency Raman spectra of Na-and Cs-DNA water solutions have been studied to determine the mode of counterion vibrations with respect to phosphate groups of the DNA double helix. The obtained spectra are characterized by the water band near 180 cm^{-1} and by several DNA bands near 100 cm^{-1}. The main difference between Na- and Cs-DNA spectra is observed in case of the band 100 cm^{-1}. In Cs-DNA spectra this band has about twice higher intensity than in Na-DNA spectra. The comparison of obtained spectra with the calculated frequencies of Na- and Cs-DNA conformational vibrations [*Perepelytsya S.M., Volkov S.N.* Eur. Phys. J. E. **24**, 261 (2007)] show that the band 100 cm^{-1} in the spectra of Cs-DNA is formed by the modes of both H-bond stretching vibrations and vibrations of caesium counterions, while in Na-DNA spectra the band 100 cm^{-1} is formed by the mode of H-bond stretching vibrations only. The modes of sodium counterion vibrations have a frequency 180 cm^{-1}, and they do not rise above the water band. Thus, the increase in intensity of the band 100 cm^{-1} in the spectra of Cs-DNA as compared with Na-DNA is caused by the mode of ion-phosphate vibrations.

Key words: DNA, counterion, vibrational mode, low-frequency spectra.

Under the natural conditions the structure of DNA macromolecule is stabilized by alkali metal counterions that neutralize the negatively charged phosphate groups of the double helix backbone [1]. The counterions, bonded to the phosphate groups, form a regular structure along the DNA backbone. This structure may be considered as an ion lattice. The formation and existence of such lattice should be characterized by the specific ion-phosphate vibrations. Therefore, the purpose of this work is to make an experimental observation of the DNA ion-phosphate mode, proving the existence of the ion-phosphate lattice.

The earlier calculations for DNA with alkali metal counterions [2–4] have been shown that the mode of ion-phosphate vibrations must be in the low-frequency spectra range (<250 cm^{-1}). The calculated frequency of ion-phosphate vibrations in case of Na^+, K^+, Rb^+ and Cs^+ counterions decreases from 180 to 90 cm^{-1} as counterion mass increases [3, 4]. The calculated

amplitudes of vibrations have been shown that the character of DNA conformational vibrations is different in case of light (Na^+ and K^+) and heavy (Rb^+ and Cs^+) counterions [4]. This difference is expected to be seen in the experimental spectra.

Existing experimental data has been shown that in this range of nucleic acid spectra there are modes, depending on the counterion type and concentration [5, 6]. In the spectra of poly(rI)·poly(rC) dry films a mode depending on the type of alkali metal counterion has been observed [5]. The frequency of this mode decreases from 150 to 110 and 95 cm^{-1} after substitution of K^+ counterions for Rb^+ and Cs^+, respectively. In the spectra of poly(dA)·poly(dT) dry films the mode 170 cm^{-1} has been observed [6]. The intensity of this mode reduces as concentration of Na^+ counterion decreases. However, in the DNA low-frequency spectra there are modes, characterizing internal vibrations of the double helix, such as vibrations of H-bond stretching in the base pairs and deformation of sugar rings. These modes are situated in the frequency range from 60 to 120 cm^{-1} [6–10].

For the experimental determination of the ion-phosphate mode among the modes of the DNA low-frequency spectra, the dependence of this mode on counterion type should be used. Therefore, in this work the low-frequency Raman spectra of DNA with light (Na^+) and heavy (Cs^+) counterions have been studied. As a result, the mode of ion-phosphate vibrations is observed in the spectra of Cs-DNA water solutions.

The samples of Na- and Cs-DNA are prepared using the DNA sodium salt from calf thymus with molecular weight of 10^7 a.u.m. The dry DNA is dissolved in distilled water to concentration of 0,2 % (by weight) and then is treated by ultrasound with the frequency of 22 kHz. The molecular weight of DNA after ultrasound treatment is determined using electrophoresis in agarous gel. The results show that molecular weight of DNA decreases to 10^5 a.u.m. Cs-DNA is obtained from Na-DNA, by replacing Na^+ for Cs^+ with the use of methods described in [7]. To perform the counterion exchange the salt CsCl (0.5 M) and two volumes of ethanol are added to 0.2 % solution of Na-DNA. In the alcohol solution, containing large concentration of CsCl salt, Na^+ counterions of DNA are replaced by Cs^+ counterions. The resulting mixture has been stored under temperature -20 ^{0}C during tree days. The DNA macromolecule precipitates. The counterion exchange is controlled with the help of Auger spectra of obtained samples. To remove an excess salt from the sample the procedure is repeated once again with much smaller concentration of CsCl salt (0.05 M). The obtained sediment has been dried under the ambient temperature during three days. The dry Cs-DNA is dissolved in water to the concentration 4%. The concentration of DNA in obtained water solutions is controlled by absorbtion band at 256 nm. Some additional salt (NaCl or CsCl) with the concentraion 0.5 M is added to the obtained solutions of Na- and Cs-DNA (4%).

The samples are excited by argon laser beam with wave-length 5145 Å. The real power of radiation transmitted to the sample, is about 60÷80 mW. The track of laser beam, passing through the cuvette with the sample, is parallel to the slot of monochromator (DFS-24). The volume and hight of cuvette is about 0.06 ml and 15 mm, respectively. The studied DNA low-frequency Raman spectra being less intensive, in order to increase a signal/noise ratio, the exposition time in the point is increased to 4÷8 s. As a result, the spectra of Na- and Cs-DNA water solutions are obtained within frequency range 30÷230 cm^{-1} (Fig. 1a). The DNA low-frequency spectra are recorded to have characteristic band near 100 cm^{-1}. The intensity of this band in the spectra of Cs-DNA is higher than in the spectra of Na-DNA.

To find the mode of DNA ion-phosphate vibrations, the detailed mathematical treatment of the spectra has been made. The spectra shapes are fitted by Lorenz curve. After substracting the spectra from Lorenz curve, the resulted spectra are expanded into Gaussian curves. As

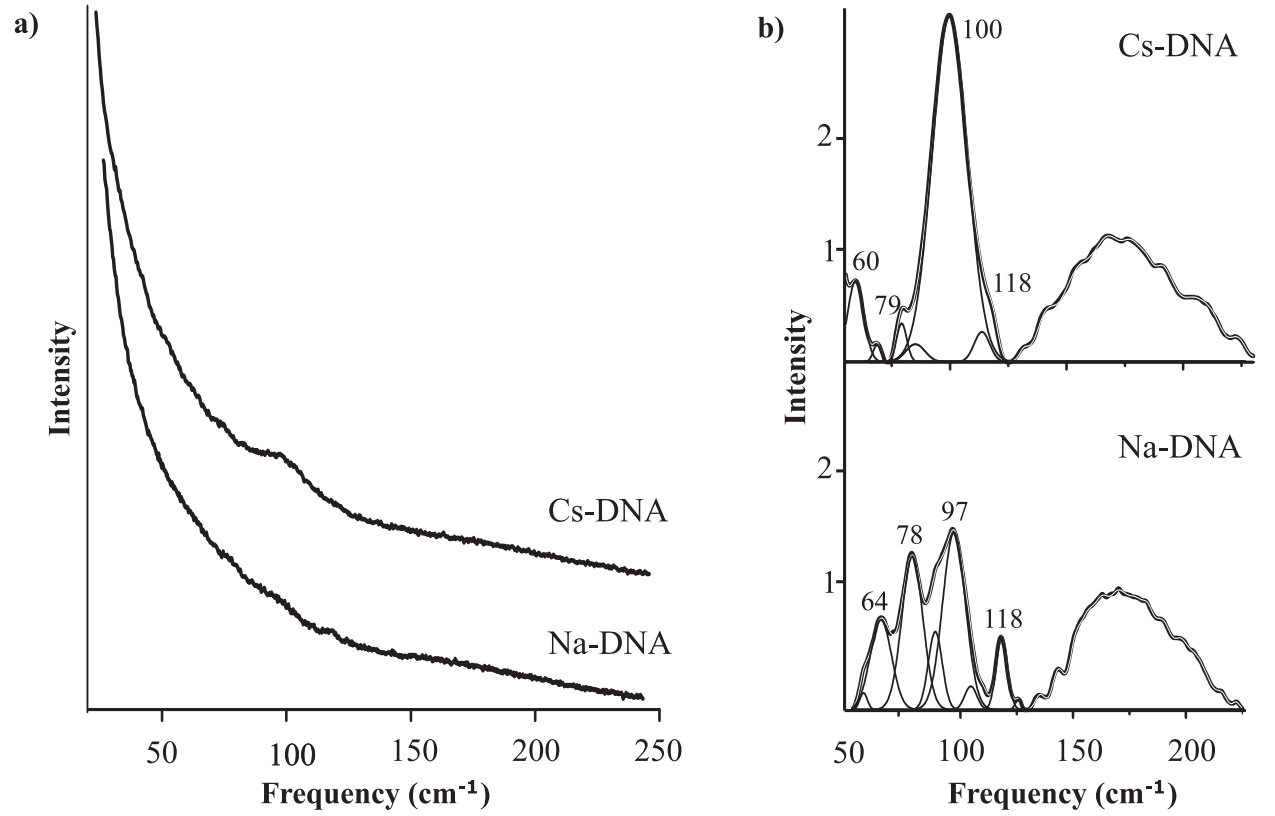

Figure 1: The low-frequency Raman spectra of Na- and Cs-DNA water solutions. The spectra before (a) and after (b) mathematical treatment.

a result, the mode frequencies are determined with accuracy ± 2 cm^{-1}. The intensities of the vibrational modes are normalized by the intensity of broad band near 180 cm^{-1}, since the intensity of this band has a similar value in both spectra. As it is known, the band 180 cm^{-1} characterizes translation vibrations of water molecules [11]. The normalized spectra of Na- and Cs-DNA are shown in figure 1b.

As one can see, within the frequency range $50 \div 120$ cm^{-1} of Na-DNA spectra the modes 78, 118 cm^{-1} and the bands 60, 97 cm^{-1} are registered. In the spectra of Cs-DNA, within the same frequency range, the modes 60, 79, 118 cm^{-1} and intensive band 100 cm^{-1} are observed. The intensity of band 100 cm^{-1} in the spectra of Cs-DNA is about twice as much than in the spectra of Na-DNA. Unlikely, modes 79 and 118 cm^{-1} are much intensive in the spectra of Na-DNA than in the spectra of Cs-DNA. At higher frequencies (more than 130 cm^{-1}) the broad band 180 cm^{-1} is observed both in the Na- and Cs-DNA spectra. The analysis of this band for selecting the DNA vibrational modes is a subject of special research, therefore, it is not discussed herein.

To determine the mode of DNA ion-phosphate vibrations the obtained frequencies of vibrations are compared with the existing experimental and theoretical data [2–9]. The frequency values and the character of DNA structural motions for observed modes are shown in table 1. The structure of obtained spectra for Na-DNA is seen to be similar to the spectra for polynucleotide dry films and for DNA crystals [6,8]. The spectra of Cs-DNA has been experimentally investigated insufficiently, however, it is known that at 95 cm^{-1} the mode depending on the

counterion type is observed [5].

The comparison of experimental data (Fig. 1b) with the calculations [2–4], has shown that the band 64 cm^{-1} in the spectra of Na-DNA characterizes the vibrations of the bases with respect to the phosphate groups of the DNA backbone (ω^+_{H+S}). These vibrations cause the deformation of sugar rings and stretching of H-bonds in the base pairs. The band 97 cm^{-1} characterizes the vibrations of bases, which cause the stretching of H-bonds (ω^+_H). According to calculations [4], the peak 79 cm^{-1} in the spectra of Na-DNA characterizes the vibrations of bases with respect to the phosphate groups of the DNA backbone (ω^+_S). These vibrations cause the deformations of sugar rings and occur without stretching of H-bonds in the base pairs. The intensity of mode 118 cm^{-1} changes after substitution of Na^+ for Cs^+ similarly to the mode 78 cm^{-1}, therefore it may characterize the deformation of sugar ring. According to calculations [4], the modes of Na-DNA ion-phosphate vibrations are located inside the band 180 cm^{-1} that characterizes the vibrations of water molecules.

Table 1. The frequencies of Na- and Cs-DNA vibrational spectra (cm^{-1}). *sh* – shoulder; *b* – band. ω^+_{ion} and ω^-_{ion} are the frequencies of ion-phosphate vibrations; ω^+_H and ω^+_{H+S} are the frequencies of H-bond stretching in base pairs; ω^-_S is the frequency of sugar ring deformations.

Na-DNA					
Our experiment(Fig. 1a)	57*sh*;64	78	88*sh*;97*b*;110*sh*	118	180*b*
Experiment [6]	63	80	95;106	–	170
Experiment [8]	68	–	96	120	–
Theory [4]	$58\omega^+_{H+S}$	$79\omega^-_S$	$110\omega^+_H$	–	$181\omega^\pm_{ion}$
Cs-DNA					
Our experiment (Fig. 1b)	–	60;69*sh*;79	85*sh*;100*b*;113*sh*	118*sh*	180*b*
Experiment [5]	–	–	95	–	–
Theory [4]	$44\omega^+_{H+S}$;	$60\omega^-_S$	$94\omega^+_H$;$103\omega^-_{ion}$;$110\omega^+_{ion}$	–	–

The calculations for Cs-DNA [3,4] show that the peak 60 cm^{-1} characterizes the vibrations of bases with respect to the phosphate backbone of DNA, causing the deformations of sugar rings (ω^+_S). The band 100 cm^{-1} is formed by the modes of H-bond stretching vibrations (ω^+_H) and ion-phosphate vibrations. In the spectra of Cs-DNA the mode ω^+_{H+S}, characterizing the vibrations of H-bond stretching and deformations of sugar rings, should be at 44 cm^{-1} [2–4]. This frequency is beyond the studied spectra range. The intensities of modes 79 and 118 cm^{-1} in the spectra of Cs-DNA are much smaller than in the spectra of Na-DNA. These modes appear in the spectra of Cs-DNA, since in the samples of Cs-DNA there are residual Na^+ ions remaining after the counterion exchange.

The analysis of obtained experimental data shows that in the spectra of Cs-DNA the modes of ion-phosphate vibrations and the modes of H-bond stretching in the base pairs form the band 100 cm^{-1}. The intensity of this band in the spectra of Cs-DNA is about twice higher than in the spectra of Na-DNA (Fig. 1b). The modes of Na-DNA ion-phosphate vibrations are situated at higher frequencies (180 cm^{-1}), and they are not separated from the wide water band. To explain why not Na-DNA, but Cs-DNA ion-phosphate modes are observed the ratio between mode intensities in the spectra of Na and Cs-DNA should be studied.

As it is known the intensity of Raman active mode is proportional to squared polarizability derivative with respect to normal coordinate [12]. The calculations [4] show that Na-DNA ion-phosphate modes are characterized by vibrations of sodium counterions solely. In the case of Cs-DNA these modes are characterized by large displacements of all structure elements of

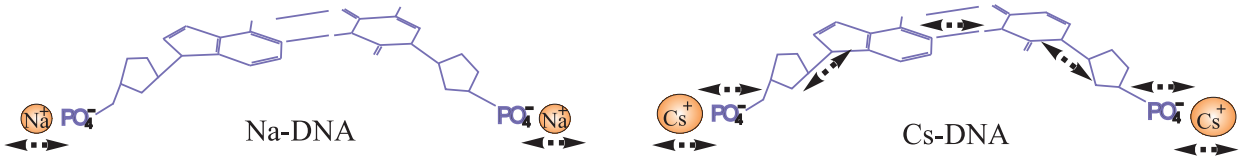

Figure 2: The displacements of the structural elements in base pair in case of ion-phosphate vibrational mode. The arrows indicate the directions of atomic group displacements. The diagrams were built using the calculation data [4]

the double helix (Fig. 2). Hence, in the case of Cs^+ counterions the displacements of the DNA structural elements should cause large changes of polarizability of the whole system. Therefore, the intensity of ion-phosphate modes in the spectra of Cs-DNA has to be much higher than in the spectra of Na-DNA. This fact explains an increase in intensity of the band 100 cm^{-1} in the spectra of Cs-DNA as compared with Na-DNA.

To summarize the results of this study it should be noted that the mode of ion-phosphate vibrations is observed in Cs-DNA Raman spectra for the first time. This mode takes place within the band 100 cm^{-1}, intensifying this band about twice. The observation of the mode of counterion vibrations with respect to the phosphate groups of the double helix verifies the existence of the DNA ion-phosphate lattice that is important for understanding of the mechanisms of DNA biological functioning.

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References

[1] *Saenger W.* Principles of nucleic acid structure, (New York: Springer-Verlag, 1984).

[2] *Perepelytsya S.M., Volkov S.N.* Ion mode in the DNA low-frequency vibration spectra // Ukr. J. Phys. **49**, 1072-1077 (2004).

[3] *Perepelytsya S.M., Volkov S.N.* Ion-phosphate vibrations of DNA in *B*- and *A*-forms // Biophysical bulletin. **1 (15)**, 5-10 (2005).[Kharkov]

[4] *Perepelytsya S.M., Volkov S.N.* Counterion vibrations in the DNA low-frequency spectra // Eur. Phys. J. E. **24**, 261-269 (2007).

[5] *Weidlich T., Powell J.W., Genzel L., Rupprecht A.* Counerion effects on the far-IR vibrational spectra of poly(rI)·poly(rC) // Biopolymers. **30**, 477-480 (1990).

[6] *Powell J.W., Edwards G.S., Genzel L., Kremer F., Wittlin A., Kubasek W., Peticolas W.* Investigation of far-infrared vibrational modes in polynucleotides // Phys. Rev. A. **35**, 3929-3939 (1987).

[7] *Weidlich T., Lindsay S.M., Rui Qi, Rupprecht A., Peticolas W.L., Thomas G.A.* A Raman study of low frequency intrahelical modes in *A*-, *B*-, and *C*- DNA // J. Biomolec. Struct. Dyn. **8**, 139-171 (1990).

[8] *Lamba Om P., Wang A. H.-J., Thomas G. J., Jr* Low-frequency dynamics of crystals, of *B*-, *A*-, and *Z*-DNA, and fibers of C-DNA // Biopolymers. **28**, 667-678 (1989).

[9] *Volkov S.N., Kosevich A.M.* Theory of low-frequency vibrations in DNA macromolecules // J. Biomolec. Struct. Dyn. **8**, 1069-1083 (1991).

[10] *Volkov S. N.* Conformation dependence of the DNA low-frequency vibrations // Biopolimery i Kletka. **7**, 40-49 (1991).[Kiev]

[11] *Urabe H., Sugawara Y., Tsukakoshi M., Ikegami A., Iwasaki H., Kasuya T.* Raman spectroscopic study on low-frequency collective modes in self-associates of guanosine monophosphates // Biopolymers. **26**, 963-971 (1987).

[12] *Carey P.R.* Biochemical applications of Raman and resonance Raman spectroscopies, (New York: Academic Press, 1982).